DEVELOPMENT OF PERFUSION PROCESSES FOR mAb PRODUCTION AIMING AT HIGH CELL DENSITIES SUSTAINED BY LOW CELL-SPECIFIC PERFUSION RATES

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Perfusion processes have been employed for approximately 25 years in the biopharmaceutical industry for the production of labile biotherapeutics, at high dilution rates and low residence times. In the last 5 years, due to the higher volumetric productivities, more compact bioreactors and possibility of integration to continuous downstream processing, perfusion has become increasingly popular also for the production of stable biopharmaceuticals, such as monoclonal antibodies (mAbs), thus allowing for process strategies involving higher residence times in the bioreactors. Viable cell densities (VCD) higher than 100 million cells per mL have been reached in the last years in perfusion processes using tangential filtration systems, such as TFF and ATF, as cell retention devices. However, these very high cell density cultures have usually been achieved through the application of high dilution rates, requiring large medium preparation facilities, large footprint for medium and harvest storage, and large capture chromatography systems to process the harvested supernatant.

The aim of the present study was to develop perfusion processes for stable biotherapeutics operating at low cell-specific perfusion rates, as a way to minimize liquid handling in perfusion-based plants. The CHO-DP12 cell line (ATCC, USA) secreting a humanized mAb was used as model system. Cells were cultured in stirred-tank bioreactors, and processes based on two cell retention devices were evaluated: a CS10 inclined lamella settler (Biotechnology Solutions, USA) and an ATF2 (Repligen, USA). A chemically defined, animal-derived component free medium (TC-LECC, Xell AG, Germany) was used as basal medium. In some runs, a concentrated nutrient solution commercialized to be used as feed for fed-batch processes (TCX2D, Xell AG, Germany) was used to fortify the basal medium, aiming at a reduction of the cell-specific perfusion rate (CSPR).

In a one-month perfusion process using the inclined settler and fed with basal medium, steady states lasting one week each were established at CSPRs progressively decreasing from 46 to 18 pL/cell/day. This allowed to sustain a VCD of approximately 50 million cells per mL at viabilities >90% at a dilution rate (harvest + bleed) of approximately 0.9 vvd. Under these conditions, a metabolic shift towards lactate consumption was observed, and an increase in mAb titer was achieved for the lower CSPRs. Based on these results, the same feeding strategy (basal medium alone, using a CSPR of approximately 18 pL/cell/d) was employed in a one-month ATF-based perfusion process with the aim of establishing very high cell density processes. A peak VCD of 136 million cells per mL was obtained, and a smooth perfusion operation at an average of 120 million cells per mL was successfully established, with viabilities higher than 87% over the entire run (Fig. 1). In comparison with the pioneering work by Clincke et al. (2013, doi: 10.1002/btpr.1704), the CSPR used in the present work is approximately 3-fold lower than theirs, and no issues regarding pumping the cell suspension in and out of the ATF cartridge were experienced, in spite of the similar high cell densities achieved. In a further attempt to decrease even more the CSPR, we investigated a gradual fortification of the basal medium by adding a concentrated feed solution in increasing proportions (up to 40% by volume), which enabled a reduction of CSPR down to 13 pL/cell/d in an ATF-based perfusion run kept at 40 million cells per mL for over 30 days. However, a progressive decrease in cell viability, especially at the highest nutrient concentrations tested, was observed, being possibly associated to the osmolality increase caused by the gradual medium fortification. Overall, these results have shown the feasibility of achieving very high cell densities at CSPRs below 20 pL/cell/d using basal medium only, and further studies are currently ongoing to further improve the process.

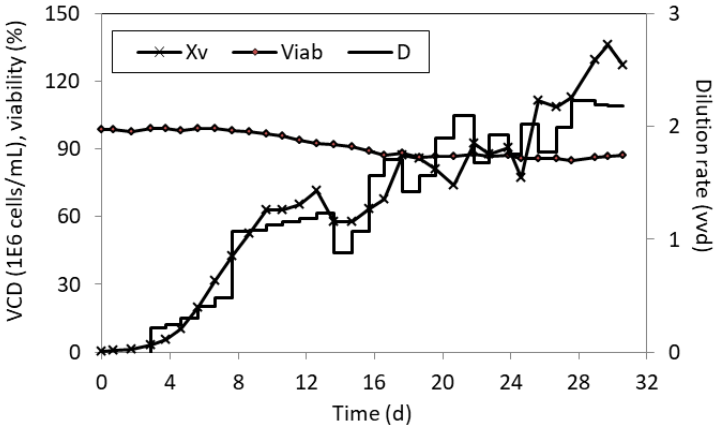

Fig. 1 - ATF-based perfusion fed with basal medium.